Original paper

CAG polymorphism in the *androgen receptor gene* in women may be associated with nodulocystic acne

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Abstract

Introduction: Acne vulgaris (AV) is a multifactorial, inflammatory disease of the pilosebaceous unit. Hormones play a major role in the pathogenesis of acne. In cases of hyperandrogenism; hirsutism, acne, seborrhoea and alopecia appear in women. However, severe acne can also be seen without evidence of hyperandrogenism. In this case, hypersensitivity of the *androgen receptor gene* (ARG) encoded in the X chromosome, which is the only receptor for androgens, can be considered. ARG contains a polymorphic CAG triple loop encoding the polyglutamine pathway at the 5'end of exon 1.

Aim: To investigate CAG repeat polymorphism in the ARG in nodulocystic acne patients in Turkish population.

Material and methods: This prospective clinical study was conducted between 2016 and 2017 in accordance with the tenets of the Declaration of Helsinki. DNA isolation from blood was performed using the RTA® Genomic DNA Isolation Kit. The fragment lengths obtained from the device to determine CAG repeat numbers were analysed based on –288 bp length 22 CAG repeat content.

Results: A total of 199 subjects; 100 patients (51 males, 49 females) and 99 controls (49 males, 50 females) were included in the study. The mean allele length in the patient group was 19.34; and 19.7 in the control group. There was a statistically significant difference between female patients and the control group, when the patients and control groups were compared by gender ($p = 0.0059$).

Conclusions: The CAG trinucleotide repeat count in the *ARG* may be associated with acne, without hirsutism findings.

Key words: acne, polymorphism, androgen receptor gene, CAG.

Introduction

Acne vulgaris (AV) is a multifactorial, inflammatory disease of the pilosebaceous unit [1]. It usually starts in puberty as a marker of puberty [1]. Hormones play a major role in the pathogenesis of acne. In cases of hyperandrogenism; hirsutism, acne, seborrhoea, alopecia appear in women [2]. However, severe acne can also be seen without evidence of hyperandrogenism. In this case, hypersensitivity of the *androgen receptor gene* (*ARG*) encoded in the X chromosome, which is the only receptor for androgens, can be considered. *ARG* encodes a protein of 910 amino acids and is highly polymorphic [3]. Androgens activate the receptor when bound to the hormone binding site, leading to nuclear translocation of the ligand-receptor complex and a number of molecular events leading to transactivation of genes regulating androgen [4]. *ARG* contains a polymorphic cytosine-adenine-guanine (CAG) triple repeat encoding the polyglutamine pathway at the 5'end of exon 1. The number of polyglutamine repeats is inversely proportional to the transcriptional activity of androgen receptor (AR) [4–6]. In this case, it may be expected that CAG repeat count is inversely proportional to AR activity.

The CAG and CGN polymorphisms, which cause sensitivity in AR had been investigated in a small number of genetic studies [7–9].

Aim

We aimed to investigate CAG repeat polymorphism in the *ARG* in nodulocystic acne patients in Turkish population.

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Material and methods

This prospective clinical study was conducted between 2016 and 2017 in accordance with the tenets of the Declaration of Helsinki. The trial protocol was approved by the Local Ethical Committee of the Kırıkkale University. Trial registration was requested on 3 March 2016 (decision no. 12/04). All patients and control subjects voluntarily participated in the study and signed an informed consent form prepared according to the ethical protocol.

A total of 199 subjects; 100 patients (51 males, 49 females) and 99 controls (49 males, 50 females) were included in the study. According to the global acne scoring system, patients with severe and very severe acne were included in the study. Patients who have used any medicines during the application or before the onset of acne were excluded from the study [10]. The women with abnormal luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, dehydroepiandrosterone sulphate (DHEA-S), progesterone, prolactin, estradiol, cortisol, adrenocorticotropic hormone (ACTH), thyroid hormone (thyrotrophin-stimulating hormone (TSH), T4, T3, free thyroxine (fT4), free triiodothyronine (fT3)) and hirsutisms findings (menstrual irregularity, hypertrichosis, seborrheic skin structure) were also excluded from the study. DNA isolation was performed using the RTA® Genomic DNA Isolation Kit. After isolation, DNA concentrations were measured by spectrophotometric methods and then stored at –20°C until use. Polymerase chain reaction (PCR) products controlled by gel electrophoresis were analysed on the ABI PRISM 310 Genetic Analyzer. The fragment lengths obtained from the device to determine CAG repeat numbers were analysed based on –288 bp length 22 CAG repeat content [11].

Table 1. Allelic distributions of males and females in patient and control groups

CAG repeat count	Male patient group, number of alleles (total 51)	Male control group, number of alleles (total 49)	Female patient group, number of alleles (total 96)	Female control group, number of alleles (total 100)
6		1		
7				
8				
9				
10				
11				
12				
13	1	2	2	
14			3	1
15	1		6	2
16	2	6	9	4
17	6	5	15	11
18	5	6	13	11
19	5	6	9	10
20	9	7	11	19
21	7	5	10	14
22	4	9	7	14
23	4	1	3	8
24	3		3	4
25	1		2	1
26	2		1	1
27		1		
28	1		1	
29				
30			1	

Ethics

The trial protocol was approved by the Local Ethical Committee of the Kırıkkale University. Trial registration was requested on 3 March 2016 (decision no. 12/04).

Statistical analysis

Student *t* test was performed using Microsoft Office Excel 2007 to compare CAG repeat count between patient and control groups. $P < 0.05$ was regarded as statistically significant.

Results

The study included 100 cases of acne (51 males, 49 females) and 99 controls (49 males, 50 females). The *ARG* is located on the X chromosome, so one allele in males and two alleles in females were detected. One woman was excluded from the study because 3 alleles were found. In Table 1, allelic distributions of males and females were given in patients and control groups. The allele range was 13–28 in males and 13–30 in females in the patients group. In the control group, it was 6–27 in males and 14–26 in females. The mean allele length in the patients group was 19.34 (20.22 in males and 18.88 in females) and 19.7 in the control group (19.18 for males and 19.96 for females). No statistically significant difference was found between patients and control groups when sex was not taken into account ($p > 0.05$). However, there was a statistically significant difference between female patients and the control group, when the patients and control groups were compared by gender ($p = 0.0059$). Since the CAG repeat number was polymorphic, except for one female patient, two alleles (one short and one long) were detected. When short and long alleles in women were compared separately in patients and control groups; there was a statistically significant

difference between short alleles ($p = 0.0006$) and no significant difference was found in long alleles ($p = 0.06$). In this case, it was concluded that the low number of repetitions in the female patients group made meaning over the short allele.

Discussion

The role of androgens in sebaceous glands and acne pathophysiology has been supported by long-standing clinical and experimental observations. The association of DHEA-S levels in the circulation during the adrenarche is known to be related to the onset of microcomedonal acne in prepubertal children [12]. There is a positive association between acne formation in small children with congenital adrenal hyperplasia or virilizing tumours [13]. The absence or the rarity of acne in men who have undergone premature castration before puberty or people with androgen insensitivity syndrome, reveals the role of androgens in acne aetiology [14]. There is a positive correlation between systemic/topical androgen or anabolic steroid treatments [15] and serum androgen levels and the number of acne lesions in women [16]. However, in women without hyperandrogenism, nodulocystic acne and androgenic alopecia can occur. This may be due to androgen sensitive receptors. Testosterone and dihydrotestosterone (DHT) act through a single nuclear receptor called AR [13]. The AR is present in epidermal and follicular keratinocytes, sebocytes, sweat gland cells, dermal papilla cells, dermal fibroblasts, endothelial cells and genital melanocytes [17, 18]. The AR number and sensitivity are ligand-dependent and are regulated by genital skin fibroblasts and sebocytes [17, 18]. The *N*-terminal region of the AR comprises of a polyglutamine sequence encoded by repeats of polymorphic CAG of variable length and a polyglycine sequence encoded by the GGN repeat. It has been shown that changes in repeat lengths in the case of both repeat types are associated with fine modulation of the AR wide expression and that various downstream targets result in modified transcriptional activity. In the normal allele range, there appears to be an inverse correlation between the CAG repeat length and the androgen wide expression; shorter alleles exhibit higher activity [19]. Considering all available data, it can be assumed that CAG repeat polymorphism of the *ARG* should play a role in acne susceptibility. In vitro results suggest that acne patients with fewer CAG repeat may display a higher AR mRNA and protein expression, which leads to a higher androgen sensitivity than control individuals. However, a study by Sawaya and Shalita found no statistically significant difference in mean CAG repeat length in acne patients and control group. However, male patients were more likely to carry the shorter repeat lengths (average length of alleles: 20) [8]. In our study, when the patient and control groups were compared by gender, we could not find any difference between patients and control groups in respect of CAG repeat count in *ARG* in males. However the number of CAG repeat in the female acne patients was lower than in the female control group ($p = 0.0059$). Yang *et al.* [9] found that male Chinese Han acne patients had a statistically less CAG recurrence than the control group, but did not find such a difference in women. Pang *et al.* [20] reported that, in contrast to the results of Yang *et al.* [9], the study of Chinese populations in north-east China revealed that CAG repeat counts for both men and women were lower in the acne group. In this study, fewer than 23 CAG repeat in men; fewer than 24 CAG repeat in women increased the likelihood of getting acne [20]. In these studies and in our study, the reasons for the differences in CAG repeat polymorphism study results in the *ARG* are unknown. Yang *et al.* [9] argued that this could reflect differences in ethnic groups. Alternatively, Sawaya and Shalita [8] have studied on a limited number of controls and acne patients, composed of individuals with diverse ethnic backgrounds (Hispanic, African and Caucasian). They argued that it was difficult to interpret the results, as average CAG repeat length of the *ARG* differs among ethnic groups [8].

When CAG trinucleotide repeats were examined, it was found inconsistent with hirsutism [21, 22]. During adolescence, androgens increase growth and sebum production in sebaceous glands more moderately in men than in women [21]. In our study, CAG repeat count was significantly lower in female nodulocystic patients without hormonal imbalance and hirsutism findings, than in women of the control group. The CAG trinucleotide repeat count in the *ARG* may be associated with acne, without hirsutism findings.

In our study, there was no correlation between CAG repeating the *ARG* and acne in males. Levels of LH, FSH, testosterone, DHEA-S, progesterone, prolactin, estradiol, cortisol, ACTH and thyroid hormones were investigated in a study and there was no relationship between serum hormone levels and acne severity in male AV cases [23]. This finding suggests that men androgen hormone levels or *ARG* CAG repeat polymorphism have no effect on acne aetiology.

Whether CAG polymorphism in the *ARG* has a causal role in the nodulocystic acne requires validation by studies in larger series and in different ethnic groups.

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Conflict of interest

The authors declare no conflict of interest.

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